dimensional materials (Vossmeyer et al., *J. Appl. Phys.* **1998**, *84*, 3664). Moreover, the ability to organize one or more types of nanoparticles into superlattice structures (Murray et al., *Science* **1995**, *270*, 1335) would allow for the construction of completely new types of hybrid materials with new and potentially interesting and useful properties.

DNA is the ideal synthon for programming the assembly of nanoscale building blocks into periodic two- and three-dimensional extended structures. The many attributes of DNA, which include ease of synthesis, extraordinary binding specificity, and virtually unlimited programmability by virtue of nucleotide sequence, can be exploited for the use of QD assembly.

The modification of QDs with DNA has proven to be more difficult than for gold nanoparticles. The common methods for preparing highly luminescent CdSe QDs yield materials that are coated with a mixture of trioctylphosphine oxide (TOPO) and trioctylphosphine (TOP). As a result, these QDs are soluble only in non-polar solvents, making them difficult to functionalize with highly charged DNA strands by direct reaction. This difficulty has been overcome by the method described below, which is the first successful modification of semiconductor nanoparticles with single-stranded DNA. It should be noted that others, in elegant studies, have looked at the interactions between QDs and duplex DNA, but these studies did not make use of the sequence specific binding properties of DNA to direct the assembly of extended QD structures. Coffer et al., *Appl. Phys. Lett,* **1996**, *69*, 3851; Mahtab et al., *J. Am. Chem. Soc.*, **1996**, *118*, 7028.

Since the surface of CdSe/ZnS core/shell QDs binds organic thiols, it was desired to modify these semiconductor particles with alkylthiol-terminated DNA strands by a substitution reaction. The lack of water solubility of these QDs, though, hindered such an approach. Two different methods recently have been reported for making QDs water soluble, allowing for the immobilization of protein structures on the QD surfaces. One involves encapsulation of the core/shell structures with a silica layer (Bruchez et al., *Science* **1998**, *281*, 2013), while the other utilizes mercaptoacetic acid both to stabilize the particles and provide water solubility (Chan et al., *Science* **1998**, *281*, 2016). The procedure described in

this example, which produces remarkably stable colloid under DNA hybridization conditions, utilizes 3-mercaptopropionic acid to passivate the QD surface.

An excess of 3-mercaptopropionic acid (0.10 mL, 1.15 mmol; Aldrich) was added by syringe to a suspension of ~20 mg of TOP/TOPO stabilized CdSe/ZnS QDs (prepared as described in Hines, et al., *J. Phys. Chem.* **1996**, *100*, 468) in 1.0 mL of N,N,-dimethyl formamide (DMF; Aldrich) generating a clear, dark orange solution containing 3-mercaptopropionic acid functionalized QDs. The reaction occurred quickly. For subsequent reactions, excess 3-mercaptopropionic acid was not removed, and the particles were stored at room temperature in DMF.

However, to characterize the QDs, a portion of the sample was purified by removing unreacted 3-mercapto-propionic acid as follows. A 0.50 mL sample was centrifuged (4 hours at 30,000 rpm), and the supernatant was removed. The remaining solution was washed with ~0.3 mL of DMF and recentrifuged. This step was repeated two additional times before recording the FTIR spectrum . FTIR (polyethylene card, 3M):1710 cm^{-1} (s), 1472 cm^{-1} (m), 1278 cm^{-1} (w), 1189 cm^{-1} (m), 1045 cm^{-1} (w), 993 cm^{-1} (m), 946 cm^{-1} (w), 776 cm^{-1} (m), 671 cm^{-1} (m). Unlike the TOP/TOPO stabilized native QDs, the 3-mercaptopropionic acid modified QDs exhibited a characteristic ν_{CO} band at 1710 cm^{-1} for the surface bound propionic acid.

Although the 3-mercaptopropionic acid modified QDs were practically insoluble in water, their solubility could be significantly enhanced by deprotonating the surface bound mercaptopropionic acid sites with 4-(dimethylamino)pyridine (DMAP; Aldrich) as described in the next paragraph. The QDs then dispersed readily in water, producing orange solutions that were stable for up to a week at room temperature.

To attach oligonucleotides to QDs, 150 μL (optical density at 530 nm = 21.4) of a solution of the 3-mercaptopropionic acid functionalized particles in DMF were added to a solution of DMAP (8.0 mg, 0.065 mmol) in 0.4 mL of DMF. An orange precipitate was formed. It was separated by centrifugation (~30 seconds at 3000 rpm) and then dissolved in 1.0 mL of a solution of 3' propylthiol- or 5' hexylthiol-terminated oligonucleotides (1.0-

2.0 ODs/mL; prepared as described in Example 1; sequences given below). Precipitate (dissolved in water) was characterized by IR spectroscopy (polyethylene card, 3M). IR (cm^{-1}): 1647 (m), 1559 (s), 1462 (m), 1214 (w), 719 (w), 478 (s). After standing for 12 hours, the oligonucleotide-containing solution was brought to 0.15 M NaCl, and the particles were aged for an additional 12 hours. The NaCl concentration was then raised to 0.3 M, and the mixture was allowed to stand for a further 24-40 hours before dialyzing against PBS (0.3 M NaCl, 10 mM phosphate buffer, pH 7, 0.01% sodium azide) using a 100 kDa membrane (Spectra/Por Cellulose Ester Membrane). The dialysis was carried out over a period of 48 hours, during which time the dialysis bath was refreshed three times.

Oligonucleotide-QD conjugates prepared in this manner displayed indefinite aqueous stability. Moreover, the colloid remained strongly fluorescent, with a sharp [full width at half maximum (FWHM) = 33 nm], symmetrical emission at 546 nm (indicative of a ~3.2 nm CdSe core; Murray et al., *J. Am. Chem. Soc.* **1993**, *115*, 8706).

Two different oligonucleotide-QD conjugates were prepared by this protocol and stored in PBS. One was modified with a 22mer, comprised of a propylthiol functionality at the 3'-end, a 12mer capture sequence, and an intervening 10 base (all A) spacer: 5'-TCTCAACTCGTAA$_{10}$-$(CH_2)_3$-SH [SEQ ID NO: 46]. The other employed a 5'-hexylthiol-terminated sequence, also with a 10 base (all A) spacer, and a 12mer capture sequence which was non-complementary with the 3'-propylthiol sequence: 5'-SH-$(CH_2)_6$-A$_{10}$CGCATTCAGGAT-3' [SEQ ID NO: 47].

C. Preparation Of QD Assemblies

When approximately equal quantities of these two oligonucleotides (200μL, OD_{530}=0.224 and 0.206, respectively) were mixed and then combined with 6μL (60 pmol) of a solution of a complementary linking 24mer sequence (5'-TACGAGTTGAGAATCCT-GAATGCG-3', SEQ ID NO: 48), QD assemblies formed within 20-30 minutes at room temperature, Figure 26. Faster linking took place when the mixture was frozen (-78° C) and then allowed to warm slowly to room temperature.